

Translation of Reference 2:

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Cloning of autolytic enzyme gene in carious bacteria

Object: Autolytic enzyme produced by bacteria is involved in metabolic turnover of cell wall and has important physiological function essential in bacterial growth, mitosis and separation. We recently isolated a gene coding an autolytic enzyme from chromosomal gene of *Streptococcus mutans* and report it.

Materials and Methods: We prepared a clone bank, wherein complete Sau3AI digested fragments of chromosomal DNA of *S. mutans* Xc strain were integrated in an integration vector. The transformed strain by the clone bank was seeded on agarose plates containing heat-killed *S. mutans* cells, and then mutants without autolytic activity were selected. Furthermore, molecular weight of autolytic enzyme was determined by Zymography.

Results and Discussion: Nucleotide sequence analysis of the integration vector-insert region of an autolytic activity-deficient mutant obtained by the above manipulation showed a mutation on a gene coding a protein with deduced molecular mass 107 kDa containing 979 amino acid residues. We prepared a mutant strain (Xc-AT') inactivated by the insertion of the present gene and compared Xc strain with Xc-AT' strain by Zymography. According to the results, crude enzyme fraction of Xc strain showed clear bacteriolytic bands at 100kDa and 80 kDa, while Xc-AT strain showed no bacteriolytic bands. Furthermore, observation of both strains under a microscope after gram staining showed that Xc-AT strain formed much longer chain than Xc strain. Therefore, it was suggested that the present gene coded a main autolytic enzyme in *S. mutans*.

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